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Full Length Research Paper

Cassava cyanocarbohydrate metabolism and proposed prehistoric symbionts

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Plant/animal symbiosis produces interacting plant food parts that bear selective pressure to produce specific behaviors that aid the plant's reproduction and/or survival. The cassava plant is here proposed to have created such a transactional food part in the cyanogenic carbohydrate tubers that acts through a sirtuin-activating pathway. The effect of exclusive cassava cyanocarbohydrate metabolism at the cellular level is to utilize the methylglyoxal bypass of glycolysis. In doing so, deleterious triose-phosphate generated methylglyoxal is dismutated to metabolites necessary for simultaneous glycolysis (inorganic phosphate and NAD). That steady high rate of NAD supply drives gradual systemic sirtuin-activation on an exclusive cassava root diet as it progresses over 1 to 7 days. Clearing the large intestine of other foods (circa 5 to 7 days) correlated with maximal expression of the Sirt1 gene dependant increased physical activity phenotype in personal testing. A rational for such a mechanism is that proposed which involves prehistoric symbionts that coevolved with the cassava plant up until the megafauna extinctions 10,000 years ago.

Key words: Cyanogenic glucosides, methylglyoxal dismutation, cassava, symbionts, sirtuin Sirt1, colon microflora, pectin.

INTRODUCTION

The Increased Physical Activity (IPA) phenotype is a Sirt1-dependant hyperactive physical behavior seen in dietary restricted animals, sometimes interpreted as a foraging instinct in response to low–nutrition/low-energy (Chen et al., 2005; Parashar and Rogina, 2009; Weed et al., 1997; Boily et al., 2008). The cassava diet (boiled tubers, plain unfermented garri cassava meal, and herbal tea) seemed to activate the IPA phenotype in personal informal testing done many times over the last 30 years. In order to rationalize that phenomena one organized test was done using commercial laboratory testing of blood-plasma pyruvate. That testing showed the diet decreased blood plasma pyruvate level to an average of 0.3 mg/dl in 20 test of 1 to 2 weeks duration, which is the low end of the normal human reference range for blood-plasma pyruvate (0.3 to 0.7 mg/dl).

The ratio of lactate to pyruvate is commonly used to reflect the NAD/NADH ratio in the cell cytosol. A higher ratio, increased NAD or lower NADH induces sirtuin gene expression (Hwang et al., 2009; Lin et al., 2004). Pyruvate was recently shown to be a histone deacetylase inhibitor. Many cancer types apparently downregulate pyruvate as a means of silencing acetylation dependant apoptosis genes. Part of that pyruvate suppression by cancer cells is the result of utilizing aerobic glycolysis that attenuates glycolysis at pyruvate by converting it to lactate and NAD in the cytosol rather than oxidizing it for NADH generation of ATP in the mitochondria. Maintaining a low concentration of cell pyruvate is central to its defense against apoptosis, and upregulating stress resistance genes (Thangaraju et al., 2006; 2009; Elangovan et al., 2011). It is a deacetylation strategy designed to suppress multiple apoptosis genes including p53 tumor suppressor by downregulating mitochondrial function, increasing the NAD/NADH ratio and ultimately activating sirtuin genes at the cellular and organismal (tumor) level (Liu et al., 2009; Fraga et al., 2005).

Abbreviations: SCFA, Short chain fatty acid; **Daf-16,** nematode abnormal dauer formation; **IPA,** increased physical activity; **Sirt1,** human silent information regulator1; **HDAC,** histone deacetylase; **NAD,** nicotinamide adenine dinucleotide; **NADH,** reduced NAD**; NADPH,** nicotinamide adenine dinucleotide phosphate reduced; **AceCS,** human acetyl CoA synthetase.

Table 1. Cassava diet daily caloric value (*ab libitum* estimate) for 59 kg male.

Parameter	Boiled cassava tuber	Plain garri meal
Daily cassava consumption	397 g (14 oz)	397 g (14 oz)
calories	608	1358
total daily calories 1966		
protein 12 g		

Table 2. Chemical composition g/100g dry mass.

Parameter	Crude protein (g)	Dietary fiber (g)	Starch (as glucose) (g)
Boiled roots	0.91	4.14	83.68
Plain gerri	1.43	3.18	88.6

(Moorthy and Mathew 1998; Tewe, 2004).

Cyanocarbohydrate metabolism may share with cancer cells that pyruvate suppressing function (see discussion) but by a different pathway; both of which may allow for a sirtuin optimal redox ratio in the cytosol rather than further high energy production in the mitochondria. Cancer progression (colony formation, invasiveness, and metastasis) correlated with increased NAD/NADH (2 fold) and decreased pyruvate (50 to 89%) utilized for mitochondrial energy generation in a breast cancer model (Singer et al., 1995). It is tempting to speculate that the organismal Sirt1 dependant-IPA phenotype has a correlate in organismal tumor-progression kinetics based on an optimal NAD/NADH redox ratio for sirtuin overexpression.

MATERIALS AND METHODS

Daily meal plan

One meal a day (ab libitum) was taken before sunset and sleep. Boiled tubers were served in a plain herbal tea broth (mainly chamomile). Garri was added to the broth as desired or eaten by itself. No other nutrition, salt, spices, herbs, oils or condiments of any kind was added. Note the diet was not designed for adequate for growth or complete nutrition, but rather to mimic the wild cassava symbionts dietary during the rooting season (Table 1).

Daily supplements were limited to vitamins used in energy metabolism as parts of coenzymes. B-1 50 mg, B-2 50 mg, B-3 nicotinic acid 200 mg (not sirtuin inhibitor niacinamide), B-6 50 mg, B-12 225 mcg, folic acid 1000 mcg, pantothenic acid 50 mg, biotin 325 mcg, C 50 mg (Tables 2 and 3). Results were previously reported in (Golay, 2010), with permission from the publisher. More precise research-grade testing is needed to verify this purported effect of a cassava food stream on pyruvate (Table 4).

DISCUSSION

There is a major fork in the road of carbohydrate metabolism at triose-phosphate formation from fructose-1,6-diphosphate. One pathway leads to glycolysis via substrate level phosphorylation proceeding from glyceraldehyde-3-phosphate and ending at pyruvate ready for conversion to Acetyl CoA and further high-energy processing in the mitochondria. The other triose-phosphate pathway (methylglyoxal pathway - glycolysis bypass) diverges from glycolysis with the enzymatic formation of dihydroxyacetone-3-phosphate via triose-phosphate-isomerase enzyme (Figure 1). It is a low-energy (no ATP produced) and non-phosphate pathway, however it does produce (inorganic phosphate-Pi and NAD) molecules necessary for glycolysis of glyceralde-3-phosphate. Methylglyoxal catalysis from dihydroxyacetone-3-phosphate (enzymatic or non-enzymatic) is a dephosphorylation step that liberates inorganic phosphate-Pi. Methylglyoxal formation is stimulated by excess carbon and cyanide, and is inhibited by high phosphates, so an exclusive low-protein/low-phosphate cyanogenic-carbohydrate like cassava may obligately take the methylglyoxal pathway. Subsequently, cyanide-induced non-enzymatic catalysis of methylglyoxal to L-lactate reoxidizes NADH to NAD. Because methylglyoxal is reactive and very toxic to the cell, it needs to be detoxified as quickly as it arises. For example, recent research on methylglyoxal toxicity has focused on its role in underpinning ROS (reactive oxygen species) formation from methylglyoxal damaged mitochondrial proteins (Schlotterer et al., 2009; Morcos et al., 2008) and showed the imperative of its detoxification enzymatically with glyloxalase-1 in diabetes and other diseases. Methylglyoxal is primarily created from triose-phosphate catalysis at this equivocal juncture of central metabolism of glucose.

The cassava cyanocarbohydrate stimulates both methylglyoxal formation and its dismutation to useful products (Pi, NAD and L-lactate). Apparently cyanide non-enzymatically catalyzes methylglyoxal to lactate very efficiently, as a cyanide-induced utilization of the methylglyoxal pathway in rats unexpectedly produced a greater than 2 fold increase in cytosolic NAD/NADH ratio, despite a more reduced mitochondrial state (Baxter et al.,

Table 3. Amino acids present in the cassava leaf and tubers.

Amino acid	Cassava leaf	Cassava tubers
arginine		7.7
Cysteine	-	-
glycine	-	-
Histidine*	-	1.5
Isoleucine*	5.3	5.2
Leucine*	10.5	5.6
Methionine*	1	.06
Phenylalanine*	3.6	3.5
Threonine	5.1	3.8
Tryptophan*	1	.5
Tyrosine*	3.3	-
Valine*	6.8	4.5

Eggum (1970); Yeoh and Chew (1976); Yeoh (1996).

Table 4. Blood plasma pyruvate testing 2007 to 2009.

Pyruvate mg./dl				
7-9 days out on cassava diet. (14 trials)			11-14 days out on cassava diet. (6 trials)	
0.3	0.3	0.2	0.1	0.4
0.4	0.4	0.3	0.1	Average 0.2
0.2	0.9	0.6	0.1	
0.3	0.2	0.2	0.3	
0.4	0.1	Average 0.34	0.2	

Normal reference range pyruvate 0.3 to 0.7 mg./dl.

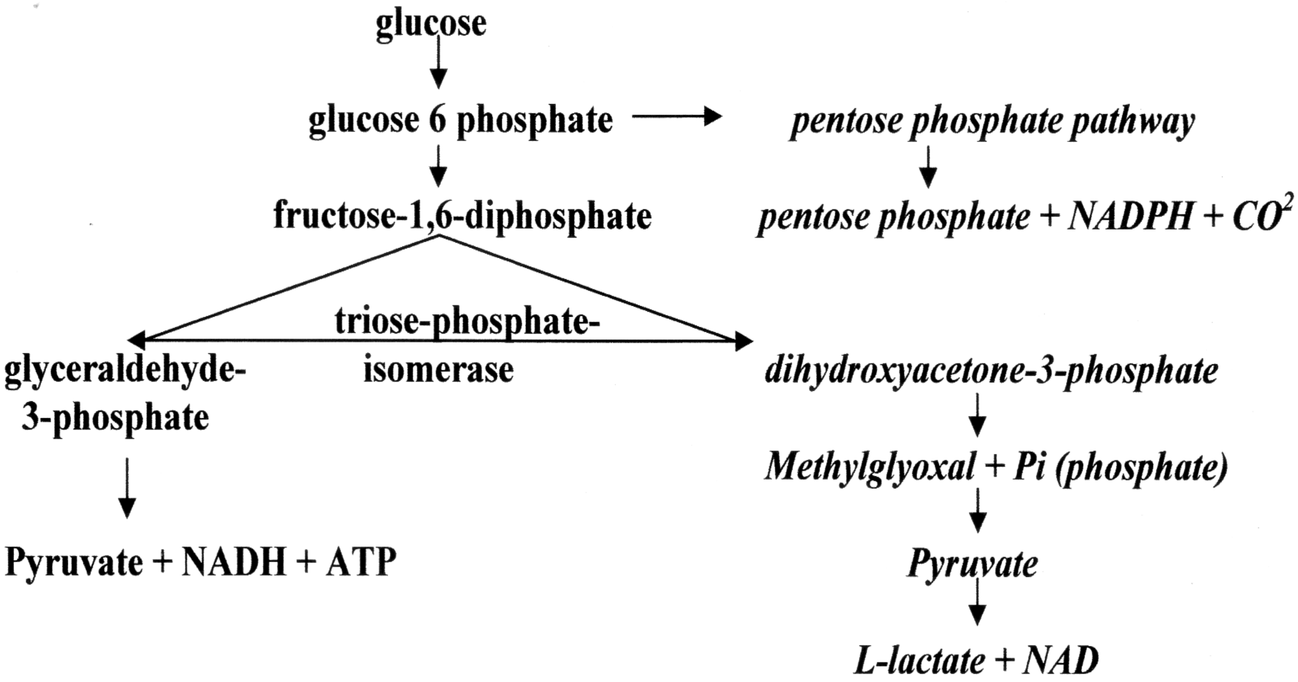

Figure 1. Cyanocarbohydrate metabolism. Carbohydrate metabolism in the presence of cyanide. Metabolic pathways induced and/or increased in utilization by cyanide in *italic.* After (Baxter and Hensley, 1969; Isom et al., 1975).

1968).

Figure 1 shows cyanide-induced or increased methylglyoxal and pentose phosphate pathways were proposed as mechanisms (Baxter and Hensley, 1969; Isom et al., 1975). Importantly, in regard to methylglyoxal enzymatic detoxification, Isom's group found a 50% reduction in glycolysis through glyceralde-3-phosphate, and a 100% increase in the pentose-phosphate-pathway that produces NADPH. NADPH is a key substrate for glutathione regeneration (reduction), a necessary cofactor for enzymatic detoxification of methylglyoxal by glyoxalase-1.

Phosphate

Phosphate is a negative regulator of carbohydrate processing via the methylglyoxal pathway as it inhibits enzymatic synthesis of methylglyoxal from dihydroxacetone-3-phosphate in bacteria (Weber et al., 2005; Ferguson et al., 1998) and goats (Ray and Ray, 1981). Conversely, low-phosphates inhibit glyceraldehyde-3-phosphate dehydrogenase enzyme, thus driving triose-phosphate metabolism (through enzymatic triose-phosphate-isomerase) to the methylglyoxal bypass with formation of dehydroxyacetone-3-phosphate and subsequently methylglyoxal formation + inorganic phosphate, which could then be used to revive stalled glycolysis. Protein-derived phosphates as inorganic phosphate - Pi, pyrophosphate - PPi, phosphoenol pyruvate - PEP, and 3-phosphoglycerate inhibit methylglyoxal synthesis 95 to 50% in the order given in bacteria. (Hooper and Cooper, 1971) High phosphates (protein foods added to the straight cassava tuber diet) would route metabolism to regular glycolysis via glyceraldehyde-3-phosphate with substrate-level phosphorylation producing pyruvate and NADH. Conversely, low phosphates (cassava tuber alone) would lead to methylglyoxal formation from dihydroxyacetone-3-phosphate and a high rate of NADH oxidation to NAD. The root is about 97 to 99% cyanocarbohydrate. The presence of high-phosphates would out-compete the non-phosphate low-energy, methylglyoxal pathway presumably induced in exclusive cassava cyanocarbohydrate metabolism. That explains why the cassava diet must be done exclusively in order to reach the low–energy level (high NADH oxidation rate) required for rapid sirtuin over-expression as evidenced by the IPA phenotype produced by a cassava tuber only diet.

Leptin and stored fat

Leptin, a hormone secreted by adipose tissue, has also been reported to control expression in the IPA phenotype in rats and anorexic humans. Anorexia nervosa patients are often hyperactive while acutely lean and consequently very low in leptin. Leptin administration was reported to negatively control the IPA phenotype in anorexia nervosa patients and semi-starvation-induced hyperactive rats (Hebebrand et al., 2003; Exner et al., 2000). Sirtuin activation, as in chronic dietary restriction or anorexia nervosa, would gradually erode adipose tissue lipids and leptin concentration through sirtuin-dependant chronic activation of acetyl-CoA synthesis from acetate derived from adipose fat (Hirshey et al., 2010). Leptin deficiency may be a downstream result of chronic sirtuin-induced long-chain fatty acid oxidation in adipose tissue. I always found that exit from the cassava diet attenuated the IPA phenotype, while my leanness was constant on or off the diet. The relationship of excess adipose tissue, leptin and Sirt1 in the IPA phenotype is unclear; however human adipose tissue contains Sirt1 and was shown to be upregulated twofold in lean women as compared to obese women. Fasting for 6 days doubled the expression of sirt1 in adipose tissue in both lean and obese women (Pedersen et al., 2008)

Sirt1 activation in the large intestine

The change in nutrition stream in the large intestine usually coincides with maximum expression of the IPA phenotype in personal testing implying that organism-wide sirtuin-activation involves deacetylation and Sirt1 activation in the large intestine. Experiments using *Caenorhabditis Elegans* nematode worms have demonstrated the centrality of the intestine in coordinating several key longevity and survival gene pathways in that organism. Low insulin/IGF-1 (insulin like growth factor) signaling, reproductive germline ablation or Sir2.1 over-expression activates Daf-16 (pro-survival gene) primarily in the intestine and secondarily in all other organs in a process called tissue entrainment (Murphy et al., 2007). The above Daf-16 activating pathways are additive in some cases, activating different and overlapping Daf-16 gene sets, achieving remarkable lifespan increases of up to four times normal in the nematode (Lin et al., 2001; Libina et al., 2003; Berdichevski et al., 2006). However, Daf-16 has not been implicated in the IPA phenotype.

Microflora generated fatty acid production in the large intestine

How a cassava cyanocarbohydrate stream remodels the intestine microflora and short chain fatty acid production (acetate, proprionate and butyrate) is unknown. However the whole tuber contains a large content of fiber at 20% (Cerada and Takahashi, 1994). Pectin is the tubers main soluble fiber (Salvador et al., 2002). High dietary pectin fiber has been reported to increase SCFA (Short Chain Fatty Acid) production by the colon microflora up to 7X over fiber-free nutrition in rats adapted to a 30% pectin diet (Stark and Madar, 1993). Pectin favors production of short chain fatty acids (SCFA) and acetate production over proprionate and butyrate, thus suppressing the two main HDAC histone deacetylase inhibitors in the colon (Jacobasch et al., 2008; Boffa et al., 1978). Short chain fatty acids butyrate ($C_4H_3O_2$), proprionate ($C_3H_6O_2$) and monocarboxylate pryruvate ($C_3H_4O_3$) are (non-sirtuin) class I HDAC (histone deacetylase) inhibitors (Thangaraju et al., 2006; 2009; Davie, 2003; Kyrylenko et al., 2003) which have been shown to cause acetylation of core histone H4K16 thus opposing Sirt1, Sirt2 and Sirt3 deacetylation of that key histone necessary for chromatin compaction and gene silencing (Scher et al., 2007; Shogren-Knaak et al., 2006).

In normal nutrition butyrate and proprionate are utilized primarily by the large intestine with the remainder being metabolized in the liver, with little escaping the liver, leading to an acetylated colon environment through histone deacetylation (HDAC) inhibition, which accumulatively affects sirtuin deacetylation status in the colon (Pruitt et al., 2006). Acetate, the dominant fatty acid produced by the colon microflora is utilized in the colon, liver and peripheral tissues. Unlike butyrate and proprionate, acetate ($C_2H_3O_2$) is not a histone deacetylase inhibitor. Pectin fiber is a complex polysaccharide combination and non-saccharide modified by methyl and acetyl esters that can be demethylated, thus making it a more preferred substrate for colon microflora. Low-methoxyl pectin is consumed faster and more completely than high-methoxyl pectins (Dongowski and Lorenz, 1998; Dongowski et al., 2002; Drochner et al., 2004). Cassava has high demethylating (methylesterase) enzyme activity (Ampe et al., 1995; Brauman et al., 1996). Pectin supplementation increased microflora populations, SCFA levels, and produced high (acetate:proprionate:butyrate) ratios, (Roy et al., 2006; Livesey and Elia, 1995; Dongowski et al., 2000) for example, (82:11:5) compared with a typical Western diet fiber ratio of (60:20:20). Pectin supplementation was positively correlated with increased thickness of the mucous layer, volume, weight, and content of stomach, small intestine, cecum and colon (Hedemann et al., 2009; Drochner et al., 2004).

High pectin supply to the colon microflora would synchronize with the full onset of the Sirt1dependant IPA phenotype when the colon is overtaken by the cassava food stream. Sirtuins (Sirt1and Sirt3) deacetylate acetyl CoA synthetase genes (AceCS1 and AceCS2), necessary for converting acetate to acetyl-CoA (activated acetate) in the cytosol and mitochondria respectively. In energetic tissue like heart and skeletal muscle the acetate is used primarily for energy production in the mitochondria TCA cycle, while in liver it is used for anabolic synthesis of cholesterol and fatty acids. (Fujino et al., 2001; Sakakibara et al., 2009) Sirtuin deacetylation induces acetyl-CoA synthesis from acetate in mammals. (Hirshey et al., 2010; Hallows et al., 2006; Shimazu et al., 2010) Similarly, bacterial sirtuin deacetylation controls Acetyl-CoA synthesis from acetate in enteric bacteria *salmonella enterica*, *Escherichia coli* and other colon microbes. (Schwer et al., 2006; Hirschey et al., 2011) It is a response to low-energy/low-nutrition that upregulates acetate metabolism, the simplest fatty acid with the lowest level of oxidizable electrons (glucose > butyrate > lactate > pyruvate > proprionate > acetate). (Livesey and Elia, 1995) Acetate supply may become a problem in a cassava diet if stored fat is absent as in anorexia nervosa, starvation, chronic dietary restriction or old age. Acetate concentration and utilization decreases dramatically in human old age (5th to 8th decade) (Skutches et al., 1979). The metabolism scheme described in Figure 1 predicts pyruvate suppression, thus making acetate-generated energy for the hyper-kinetic IPA phenotype more important. The low pyruvate would be accounted for by the non-enzymatic dismutation of pyruvate to lactate in the methylglyoxal pathway and by the simultaneous reduction by 50% of glycolysis of glyceraldehyde 3 phosphate to pyruvate as described in (Baxter and Hensley, 1969; Isom et al., 1975). As long as there is a supply of acetate, (endogenous from glucose metabolism and fatty acid oxidation or exogenous from the colon microflora and diet) then upregulated acetyl-CoA synthesis induced by Sirt1 and Sirt3 activation can supply anabolic lipids, like cholesterol and fatty acids, for cell biosynthesis in the cytosol endoplasmic reticulum, or in the mitochondria through the glyoxylate cycle, or as fuel for the TCA oxidative phosphorylation cycle. In addition, free fatty acid availability, including acetate, spares protein proteolysis, leucine oxidation and reduction in the nitrogen balance associated with protein loss in fasting and calorie restriction (Tessari et al., 1986; Bailey et al., 1993). Adequate calories (glucose), and especially acetate, may be critical for maintaining a healthy sirtuin-induced IPA phenotype for extended periods of time by preventing excessive autophagy and proteolysis that may occur in the absence of adequate acetyl-CoA preferred substrates glucose and acetate. Interestingly, acetone is a component of linamarin, the principle cyanogenic glucoside in cassava at 80-90%. The linamarin molecule breaks down to yield 1 mol each of glucose, HCN, and acetone. Acetone is metabolized to D-Lactate, L-Lactate, pyruvate and acetate by three energy inefficient processes. Formerly it was considered a waste product of metabolism. Acetone in part is converted to methylglyoxal and metabolized in the methylglyoxal pathway to pyruvate (Flowers et al., 2003; Kalapos, 2003). The effect of cyanide on acetone-generated methylglyoxal metabolism is unknown, but presumably acetone generated methylglyoxal could serve as an added substrate for further increasing the NAD/NADH ratio similar to dihydroxyacetone-3-phosphate processing in that pathway, (Figure 1). The other cyanogenic glucoside in cassava is lotaustralin, which breaks down to 1 mol. each

of glucose, HCN, and 2-butone (ethyl methyl ketone).
Of its carbohydrate, 64 to 72% is made up of starch. Cassava starch contains 20 percent amylose and 70 percent amylopectin. The raw starch of the cassava root has a digestibility of 48.3% while cooked starch has a digestibility of 77.9 % Amylase enzymes in saliva and from the pancreas converts the amylose and amylopectin into disaccharides and trisaccharides which are converted by other enzymes to glucose Tewe (2004). The remaining starch is metabolized by colon microflora to short chain fatty acids.

The cassava cyanocarbohydrate composition may have been selected for symbiotically over several million years to best fulfill the molecular requirements for rapid organismal-sirtuin-activation necessary for maximal IPA phenotype expression in a large mammal. By simultaneously evolving a food component (high pectin fiber concentration) that generates maximum acetate supply from colon microflora, the plant insured that even in an aged or very lean acetate-deficient symbiont, that there would be adequate energy and lipid synthesizing power to support the increased physical activity phenotype for as long as tubers remained to be eaten.

Cassava – *manihot esculenta* Crantz symbionts

Today humans are cassava's surrogate symbiont partners. We insure their reproduction and extend their numbers and range, while humans get a huge increase of caloric food supply, especially in underdeveloped tropical countries. It is proposed here that the ancestor species of cassava (*M. Peruviana* as the progenitor and *M. flabellifolia* as the intermediate ancestor) had several now-extinct prehistoric large mammal symbionts. The alpha symbiont was the giant ground sloth (Pujos, 2008). In this scheme the alpha symbionts were giant ground sloth foliovores (leaf eaters) that ate the very palatable high-protein (7%) leaves and seed capsules, which were dispersed after passive transit through a digestive system pre-adapted for seed sparing. A second method was physical dispersal of reproductive stalk nodes during leaf gathering with their 2 long claws. The brittle stalk would have allowed sections of stalk to be clawed to the ground where it would have been buried by the weight of the sloth and the cultivating action of its hind claws. The long non-tuberous roots originally were reserve energy storage depots for regenerating new upper-story plant stalk and leaves after over-browsing or forest fires. In this scheme giant sloths first interacted with *manihot Peruviana*, which were characteristically forest edge scandent vine-like climbers, clambering over other vegetation (to 9 m) with branch fusion and lateral networking resulting in a supportive structure suitable for high leaf production. What distinguishes the progenitor is scandent growth posture that may have enhanced leaf production for foliovore seed-dispersal synchronous with leaf ingestion as the primary large-mammal symbiont-assisted mechanism. Later when the symbiont became the massive giant ground sloth, that method continued, but the plant adapted to the giant sloth with expanded vegetative node development, which was a latent but present character from its decumbent (crawling) past. The transactional food for the giant sloth remained the leaf, but due to its size and mode of eating, vegetative reproduction from stem nodes gradually assumed the dominant mode of reproduction, perhaps because it was more efficient in generating adventitious (added from a different source and not inherent or innate) storage roots to support leaf regeneration. With the shift to symbiont-assisted vegetative reproduction came divergent speciation toward vegetative reproductive genes and associated morphological characters (node development and adventitious roots).

Pampatheres

Later a second symbiont developed that was focused on the now expanded adventitious roots. That change spurred differentiation of descendant species of the progenitor toward free-standing posture away from the forest edge, larger carbohydrate roots, larger stems, and node development more optimal for symbiont-assisted dispersal centered on the tuber as food. The beta symbiont was probably an herbivorous rooting pampatheres or glyptodont (Vizciano et al., 2008). They would have dug up and eaten the roots at maturity (circa 12 months) thus destroying the plant but in the process scattering the stalk nodes. By scattering the stalk in the immediate vicinity, and by dragging the roots with sections of attached stalks to a safer place to eat them, the range of propagation would have taken on a new dimension away from the forest edge where the giant ground sloth browsed on abundant leaves. The giant ground sloth's habitat probably came to resemble a cultivated pattern as the sunny edge of the forest became more populated with its preferred food plant. It has been proposed that these sloths were socially gregarious as fossil remains of various-age individuals were found together at one fast-flood site. (Rossetti et al., 2004) There was probably considerable tension between the two cassava symbionts, but overall, having two contiguous zones of symbiocity based on different plant food parts (leaf and root) led to mutual food security for both animals. It is noteworthy that the (sympatric) shared transition zone of extant *M. Peruviana* and *M. Flabellifolia* is also the zone now believed to be the area of first human cultivation of cassava (Figure 2).

The development of a sustaining food with a large symbiont mammal is rare in nature and probably would not have occurred without a symbiont capable of defending the local territory of vegetative symbio-cultivation. The massive size, power, restricted mobility

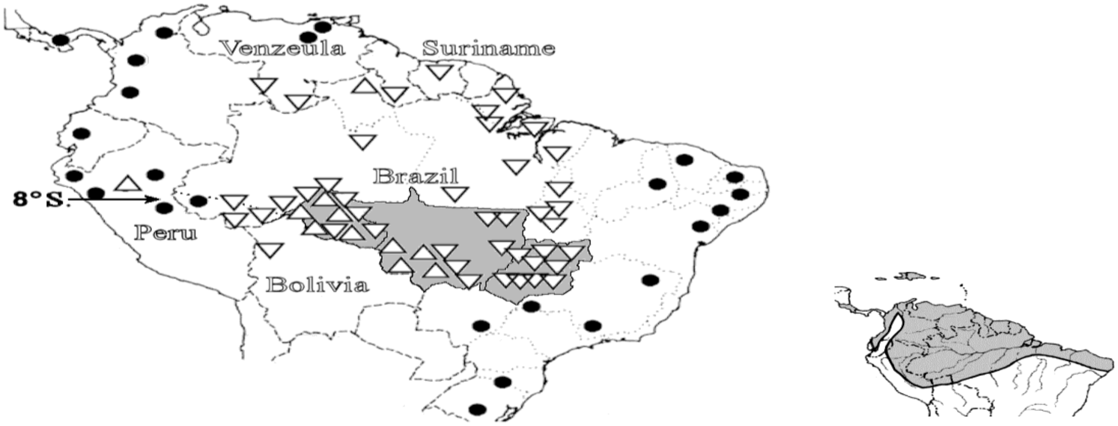

Figure 2. *E. Laurillardi* giant ground sloth fossil sites and proposed extant cassava progenitor and intermediate ancestor distribution in South America.

● *Eremotherium Laurillardi* giant ground sloth fossil sites (Pujos and Salas, 2004; Pujos, 2008; Cartelle and De Uuliis, 1995)*

△ *Manihot Peruviana* extant wild sites. (Allem, 1994; Olsen and Schall, 2001; Rogers and Appan, 1973; Allem, 1994, 2002; Olsen and Schall, 2001). *Manihot flabellifolia* extant wild sites. (Allem, 1994; Olsen and Schall, 2001). **8° S.** – Proposed entry point for *E. Laurillardi* Amazonian radiation. (Pujos et al., 2004; Pujos, 2008).

and potential longevity of the giant ground sloth was a perfect match that gave stability to a relationship that increased in dominating the forest edge over time. The cassava plant is capable of producing large quantities of carbohydrate and protein. On a calorie-per-hectare basis (leaf and tuber) it is among the most productive food plants in the world. The beginning of the Tertiary period, 65 million YBP (years before present), marked the extinction of the dinosaurs and the beginning of the age of mammals and angiosperm plants with their myriad coevolutionary symbiotic relationships. South America was isolated from North America until the gradual rising of the isthmus land bridge linking North and South America 4 million YBP. That was followed by the megafaunal extinctions of the late Pleistocene - early Holocene (2 million -10,000 YBP). The causes have been attributed to climate changes (glaciations), the Great American Interchange of species coincident with the reconnection of North and South America, and finally by human predation. It has been proposed that many specialized symbiotic relationships that thrived before that megafaunal extinction were severed, leaving only the angiosperm plant without their large mammal seed-dispersing partner (Janzen and Martin, 1982).

Giant ground sloth *Eremotherium Laurillardi*

The radiation of lowland tropical giant ground sloths *E. Laurillardi* branched off from the Andean highland giant ground sloths (both Megatherium) at about 8˚ S. latitude central Peru (Figure 2) (Pujos and Salas, 2004; Pujos, 2008). *E. Laurillardi's* dispersal probably radiated east around the southern extents of the Amazon basin and eventually around the northern extents of the basin and into tropical Central America in the Pleistocene 2 million YBP (Cartelle and De Iulius, 1995). That Panamerican distribution was the largest of any ground sloth species and eventually included southern North America, northern South America, Central America and Caribbean islands before and after the rising of the isthmus at Panama. *E. Laurillardi* weighed up to 3000 kg and had a maximum reach of about 5.5 m (18') in upright (bipedal) posture using a strong tail for support. The high point in number of species, size, and range of ground sloths occurred in the last 2 million years before the megafaunal extinctions of all ground sloths and most other megafauna herbivores world-wide except Africa, that characterized that period. The height of the extinctions coincided with radiation of humans out of Africa into all continents and strongly implicates humans in the final extinctions of these sloths (Long and Martin, 1974; Steadman et al., 2005).

E. Laurillardi had several characteristics that made them ready partners for plant symbiocity involving reproductive plant parts (stem nodes and seeds) in foliovore trade-off feeding arrangements. Long before these giant sloths met *Manihot Peruviana* in tropical eastern Peru, they probably had other symbiont relationships with sexual and vegetatively reproducing edible herbs, trees and vines.

Eremotherium Laurillardi giant ground sloth characteristics that aided *Manihot Peruviana* symbiosity

1. Its giant size with long claws allowed dominance in its local environment in a passive defensive manner. The clumsy sparse (2) claws made leaf gathering by pulling

branches down close to the mouth synchronous with occasionally knocking down sections of stalk with vegetative reproductive nodes. A long prehensile tongue may have been used for stripping leaves and bringing them into the mouth (Rossetti et al., 2004; Tito and De Uuliis, 2003) similar to extant Giraffe, which are cyano-foliovores that prefer the cyanogenic *acacia erioloba* and other acacia tree leaves collected by a long prehensile tongue.

2. Like extant Giant Panda, Red Panda, Golden Bamboo Lemur, Mountain Gorilla, Giraffe, Bamboo rats and other high-cyanide eaters, cyanide toxicity was probably dealt with by enzymatic detoxification but is largely unknown. In mammals the enzyme rhodanese detoxifies cyanide to much less toxic thiocyanate. In herbivores, both foregut and hindgut intestinal microflora hydrolyze, metabolize, and otherwise detoxify cyanogenic glucosides. (D'Mello et al., 1991), while other microbes are cyanide sensitive and growth repressed.

3. These giant ground sloths were tardigrades (slow-movers). The claws kept them from rapid mobility and enforced a restricted range for individual sloths. They are thought to have walked on the sides of their feet when not walking bipedally.

4. The dentition of *E. Laurillardi* and many other ground sloths in general, is not typical of large herbivores. They had low numbers of teeth per jaw, 5 upper teeth and 4 lower teeth per quadrant, no canine or frontal teeth, and diastema (spaces) between teeth. Occlusal (grinding) surface area was low, thus producing low food processing in the mouth and high fermentation processing of food in a fermenting foregut digestive system (non-ruminate). Taken together, that dentition may have allowed passive transit of reproductive plant parts (seeds or reproductive nodes) with manihot and other plants. Dentition characteristics would have discouraged root consumption while manus (hand) and forearm characteristics would have discouraged locomotion and root digging (Tito and De Uuliis, 2003; Pujos, 2008).

Manihot Peruviana characteristics that aided giant ground sloth symbiocity

1. High-protein palatable leaf with a nutritional profile rich enough to sustain the sloth and cyanogenic enough to resist insects. The edible leaf easily snaps loose from the petiole with slight downward pressure making it simple to acquire with a long prehensile tongue. The petiole also detaches (snaps) loose easily from the stalk. The seed capsules are produced monoeciously (both sexes on the same plant) and by cross-pollination. Seed capsules are often surrounded by leaf-like sheaths.
2. The brittle stalk has vegetative reproductive nodes.
3. The *M. Peruvianae* sect proposed by (Rogers and Appan, 1973) of morphologically similar scandent forms with non-tuberous roots covers the Amazon basin area plus Hispaniola and Costa Rica. (All 4 in the progenitor group in Table 1).
4. Long non-tuberous storage roots for vegetative regrowth of leaves.

Adventitious carbohydrate tubers are most associated with vegetative reproduction from stem nodes and appear only in the progenitor's more cassava-like descendant *M. flabellifolia* and its close relatives, (All in the intermediate group in Table 1).

Cassava *Manihot esculenta* Crantz origin

The genus Manihot lies within the family Euphorbiaceae and contains some 98 species, widely distributed throughout the New World tropics, but confined to the American continent. There are 80 species in South America and 17 species in tropical Mexico/Central America. The species *Manihot esculenta* Crantz (domesticated cassava) was considered to be a cultigen "compilospecies" developed from hybridization of several *Manihot* wild species, with no wild plants, except as escapes from cultivation. Its geographic origin and taxonomic position was obscure and argued for over 100 years with Mexico and Central America considered the probable area of origin. That opinion has changed since about 1987 when Costa Allem proposed the ancestors of *Manihot esculenta* Crantz to be several closely related wild species (*Manihot peruviana* and *Manihot flabellifolia*) originating around the southern rim of the Amazon basin from Eastern Peru thru Central Brazil based first on extensive field collection of wild species evidence (Allem, 1994; 2000; 2001; 2002). Since then numerous investigators have confirmed that proposal with molecular and genetic studies (Schall et al., 2006; Olsen and Schall, 1999; 2001; Fregene et al., 1994; Roa et al., 2000). That analysis places the above 2 wild species in a high-affinity gene pool (Table 1) that contains *M. esculenta Crantz* (the domesticate) plus *M. peruviana* and *M. flabellifolia,* with the latter two as synonym progenitor species. In the present interpretation, morphological distinctions are made between *M. Peruviana* and the more diversified (cassava-like) *M. Flabellifolia*.

Biome collapse

When the alpha and beta symbionts disappeared 10,000 years ago (Steadman et al., 2005), the plant characteristics associated with that symbiotic vegetative reproduction (nodes, carbohydrate tubers, and freestanding forms) regressed in the wild progenitor, and its descendants as those wild species reverted to strict sexual reproduction and associated primordial morphological forms for the next 10,000 years. That

Table 5. Stages of *Manihot* diversification and involved symbionts.

Cassava Gene pool 1*		**Characteristics**	**Symbiont / food part / reproductive part**
Progenitor	*M. Peruviana***	scandent climbers / long non-tuberous roots / sparse reproductive nodes with long internodes.	Giant ground sloths / leaf/seed pods and Reproductive nodes with mechanical stem dispersal.
	M. leptophylla	Extant wild reproduction – sexual (latently vegetative)	
	M. brachyloba		
	M. quinquepartita		
Intermediate	*M. flabellifolia***	Mixed scandent and free-standing posture, carbohydrate tubers / more developed stem nodes with shorter internodes	Pampatheres or glyptodont / tubers / reproductive nodes
	M. saxicola	Extant wild reproduction – sexual (latently vegetative)	
	M. pruinosa		
	M. grahami		
	M. aesculifolia		
	M. carthaginensis		
	M. pringlie		
	M. manipeba		
Cultivated	*M. esculentaCrantz**	Free-standing / large carbohydrate roots / well developed stem nodes with short internodes. Cultivated Reproduction – vegetative (latently sexual)	Humans/ root and leaf/ reproductive nodes

*(Allem et al., 2001; Allem, 2002; Olsen, 2004; Roa et al., 2000). Sect Peruvianae. All in progenitor box. (Rogers and Appan ,1973). Other intermediate species: *M. saxicola, M. pruinosa, M. grahami, M. aesculifolia, M. carthaginensis, M. pringlie, M. manipeba*

repression can be partially relieved by repeated vegetative reproduction cycles as demonstrated by the domestication of *M. saxicola,* (Lanjouw, 1939) *M. flabellifolia,* (Allem, 1994; 2000) *M. pringlie*, (Rogers and Appan, 1973) and *M. manipeba* (Allem, 2002). Either type of reproduction represses the other type through negative resource allocation over many generations and can be revived as long as those latent genetic programs exist in the species (Schall et al., 2006). The role of the disappeared symbionts may be the missing link in understanding how progenitor scandent climbers with non-tuberous roots got to be carbohydrate tubers in free-standing plants before humans replaced the natural symbiont. The explosive radiation of *E. Laurillardi* out of Peru in the Pleistocene and early Holocene is geographically and maybe temporally synchronous with the geographic range (Figure 2) of the cassava ancestors, as the genus manihot is considered to have arisen and diversified recently. That argument is based on a lack of variability in chromosome number, low levels of diversity in floral morphology, (Rogers and Appan 1973) DNA sequence data, (Olsen and Schall 1999; Fregene et al. 1994; Roa et al. 2000) and by inter-fertility between morphologically divergent species in artificial crosses. It may be that this proposed very close coevolutionary partnership with the giant ground sloth was central to morphological evolution and distribution of the genus manihot, and was an anchoring element for a secondary symbiotic dispersal relationship, both of which collapsed completely after the megafaunal extinctions. Grey area in large map.

The putative first cassava cultivation area is based on earliest agricultural archaeology, (Olsen and Schall 2001) molecular, (Olsen and Schall 1999) and phylogeographic evidence (Allem 2002; Nassar 1978). It covers the Brazilian states of Rondonia in the west, Mata Grosso and Goias in the east with Rondonia the most favored.

Grey area in small map – Peruvianae sect (Rogers and Appan, 1973) distribution (*M. Peruviana, M. Leptophylla, M. Quinquepartita,* and *M. Brachyloba*)

*Fossil sites from the Amazon lowlands are extremely rare due to the high acidy, moisture, and vegetation there degrades bone but are relatively abundant in Andean caves and desert areas Figure 1 and 2.

Conclusion

Cyanocarbohydrate metabolism in an exclusive cassava root diet may involve methylglyoxal generation and dismutation producing a high NAD/NADH ratio at the cellular level. That change in cellular redox state gradually accumulates to a multi-cellular organismal sirtuin phenotype over 1 to 7 days as the alimentary canal organs are overtaken by the sirtuin-activating food stream as evidenced by increased IPA phenotype expression. Organism-wide sirtuin-activation reaches maximal effect when the large intestine is cleared of previous nutrition. These proposed attributes of the cassava root may have been symbiotically-selected for rapid organismal sirtuin-activation of a large mammal to induce the Sirt1 dependant IPA phenotype in order to hyper-energize (increase) a work-intensive (rooting) symbiont-assisted vegetative-reproduction routine. Mimicking that nutrition may allow temporary human organismal sirtuin (Sirt1) activation.

Model organisms for testing these hypotheses may include enteric bacteria, cassava-philic nematodes, rats, miniature pigs, and primates, including humans.

REFERENCES

Allem AC (1994). The Origin of *Manihot esculenta* Crantz (Euphorbiaceae). Genet. Resour. Crop. Ev., 41(3): 133-150.

Allem AC (2000). Ethnobotanical testimony on the ancestry of Cassava (*Manihot esculenta* Crantz subsp. Esculenta). Plant Genet. Resour. Newsl., 123: 19-22.

Allem AC, Mendes RA, Salomao AN, Burle ML (2001). The primary gene pool of cassava (*Manihot esculenta* Crantz subspecies *esculenta*, Euphorbiaceae). Euphytica, 120: 127-132

Allem AC (2002). The Origin and Taxonomy of Cassava. EMBRAPA, Recursos Geneticos e Biotecnologia, 1-16.

Ampe F, Keleke S, Robert H, Brauman A (1995). The role and origin of pectin degrading enzymes during cassava retting. Transformation Alimentaire du Manioc. In Agbor Egbe T, Brauman A, Griffon D, Trèche S (eds.) 1995 edition ORSTOM

Bailey JW, Miles JM, Haymond MW (1993). Effect of parenteral administration of short-chain triglycerides on leucine metabolism. Am. J. Clin. Nutr., 58(6): 912-916.

Baxter RC, Hensley WJ (1969). The Effect of Ethanol and Cyanide on NAD/NADH2 Ratios in the Rat Liver. Biochem. Pharmacol., 18: 233-236.

Berdichevski A, Viswanathan M, Horvitz HR, Guarente L (2006). C. Elegans Sir-2.1 interacts with 14-3-3 proteins to activate Daf-16 and extend lifespan. Cell, 125: 1165-1177.

Boffa LC, Vidali G, Mann RS, Allfrey VG (1978). Suppression of histone deacetylation in vivo and in vitro by sodium butyrate. J. Biol. Chem., 253(10): 3364-3366.

Boily G, Seifert EL, Bevilacqua L, He XH, Sabourin G, Estey C, Crawford S, Saliba S, Jardine K, Xuan J, Evans M, Harper M,

McBurney MW (2008). Sirt1 regulates energy metabolism response to caloric restriction. Plos One, 3(3): e1759.

Brauman A, Keleke S, Malonga M, Miambi E, Ampe F (1996). Microbiological and biochemical characterization of cassava retting, a traditional lactic Acid fermentation for foo-foo (cassava flour) production. Appl. Environ. Microbiol., 62(8): 2854-2858.

Cartelle C, De Iuliis G (1995). Eremotherium: The Panamerican late Pleistocene megatheroid sloth. J. Vert. Paleontol., 15(4): 830-841.

Cerada MP, Takahashi M (1994). Cassava waste: Their characterization, uses and treatment in Brazil. In: Cassava flour and starches: progress in research and development. CIAT-CGIAR International Center for Tropical Agriculture – CGIAR, p. 223. http://webapp.ciat.cgiar.org/agroempresas/pdf/cassava_flour%20_session%204.pdf

Chen D, Steele AD, Linquist S, Guarante L (2005). Increase in Activity During Calorie Restriction Requires Sirt1. Science, 310: 1641.

Davie JR (2003). Inhibition of histone deacetylase activity by butyrate. J. Nutr., 133(7): 2485S-2493S.

D'Mello JPF, Duffus CM, Duffus JH (1991). Toxic substances in crop plants. Roy. Soc. Chem. Cambridge, UK. pp. 220-223.

Dongowski G, Lorenz A (1998). Unsaturated oligogalacturonic acids are generated by in vitro treatment of pectin with human faecal flora. Carbohyd. Res., 314(3-4): 237-244.

Dongowski G, Lorenz A, Anger H (2000). Degradation of pectins with different degrees of esterification by bacteroides thetaiotaomicron isolated from human gut flora. Appl. Environ. Microbiol., 66(4): 1321-1327.

Dongowski G, Lorenz A, Proll J (2002). The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro. J. Nutr., 132(7): 1935-1944.

Drochner W, Kerler A, Zacharias B (2004). Pectin in pig nutrition, a comparative review. J. Anim. Physiol. Anim. Nutr. (Berl.), 88(11-12): 367-380.

Eggum BO (1970). The protein quality of cassava leaves. Br. J. Nutr., 24(3): 761-768.

Elangovan S, Ramachandran S, Venkatesan N, Ananth S, Gnana-Prakasam JP, Martin PM, Browning DD, Schoenlein PV, Prasad PD, Ganapathy V, Thangaraju M (2011). SIRT1 is essential for oncogenic signaling by estrogen/estrogen receptor α in breast cancer. Cancer Res., 71(21): 6654-6664.

Exner C, Hebebrand J, Remschmidt H, Wewetzer C, Ziegler A, Herpertz S, Schweiger U, Blum WF, Preibisch G, Heldmaier G, Klingenspor M (2000). Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. Mol. Psychiatr., 5(5): 476-481.

Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG, Pérez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MA, Ahn N, Imhof A, Caldas C, Jenuwein T, Esteller M (2005). Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet., 37(4): 391-400.

Ferguson GP, Totemeyer S, MacLean MJ, Booth IR (1998). Methylglyoxal production in bacteria: suicide or survival. Arch. Microbiol., 170: 209-219.

Flowers L, Broder MW, Forsyth C (2003). Toxicological review of acetone (CAS No. 67-64-1). U.S. Environmental Protection Agency, Washington, D.C., http://www.epa.gov/iris

Fregene MA, Vargas J, Ikea J, Angel F, Tohme J. Asiedu RA, Akoroda MO, Roca WM (1994). Variability of chloroplast DNA and nuclear ribosomal DNA in (*Manihot Esculenta* Crantz) and its wild relatives. Theor. Appl. Genet., 89: 719-727.

Fujino T, Kondo J, Ishikawa M, Morikawa K, Yamamoto TT (2001). Acetyl-CoA synthetase 2, a mitochondrial matrix enzyme involved in the oxidation of acetate. J. Biol. Chem., 276(14): 11420-11426.

Golay VK (2010). Cassava root diet induces low pyruvate levels. Rejuvenation Res., 13(2-3): 260-261.

Hallows WC, Lee S, Denu JM (2006). Sirtuins deacetylate and activate mammalian acetyl-coA synthetases. PNAS. 103(27): 10230-10235.

Hebebrand J, Exner C, Hebebrand K, Holtkamp C, Casper RC, Remschmidt H, Herpertz-Dahlmann B, Klingenspor M (2003). Hyperactivity in patients with anorexia nervosa and in semi-starved rats: evidence for a pivotal role for hypoleptinemia. Physiol. Behav., 79: 25-37.

Hedemann MS, Theil PK, Bach Knudsen KE (2009). The thickness of the intestinal mucous layer in the colon of rats fed various sources of non-digestible carbohydrates is positively correlated with the pool of SCFA but negatively correlated with the proportion of butyric acid in digesta. Br. J. Nutr., 102(1): 117-125.

Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C, Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB, Farese RVJr, Alt FW, Kahn CR, Verdin E (2011). SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature, 464(7285): 121-125.

Hirschey MD, Shimazu T, Capra JA, Pollard KS, Verdin E (2011). SIRT1 and SIRT3 deacetylate homologous substrates: AceCS1, 2 and HMGCS1, 2. Aging (Albany NY). 3(6): 635-642.

Hooper DJ, Cooper RA (1971). The regulation of *Escherichia coli* methylglyoxal synthase; a new control site in glycolysis? FEBS Lett., 13(4): 213-216.

Hwang JH, Kim DW, Jo EJ, Kim YK, Jo YS, Park JH, Yoo SK, Park MK, Kwak TH, Kho YL, Han J, Choi H, Lee S, Kim JM, Lee I, Kyung T, Jang C, Chung J, Kweon GR, Shong M (2009). Pharmacological Stimulation of NADH Oxidation Ameliorates Obesity and Related Phenotypes in Mice. Diabetes, 58: 965-974.

Isom GE, Liu DHW, Way JL (1975). Effect of Sub-lethal Doses of Cyanide on Glucose Catabolism. Biochem. Pharmacol., 24: 871-875.

Jacobasch G, Dongowski G, Florian S, Müller-Schmehl K, Raab B, Schmiedl D (2008). Pectin does not inhibit intestinal carcinogenesis in APC-deficient Min/+ mice. J. Agric. Food. Chem., 56(4): 1501-1510.

Janzen DH, Martin PS (1982). Neotropical Anachronisms: The fruits the Gomphotheres Ate. Science, 215: 19-27.

Kalapos MP (2003). On the mammalian acetone metabolism: from chemistry to clinical implications. Biochim. Biophys. Acta., 1621(2): 122-139.

Kyrylenko S, Kyrylenko O, Suuronen T, Salminen A (2003). Differential regulation of the Sir2 histone deacetylase gene family by inhibitors of class I and II histone deacetylases. Cell Mol. Life Sci., 60(9): 1990-1997.

Lanjouw J (1939). Two interesting species of Manihot from Suriname. Rec. Trav. Bot. Neerl., 36: 543-549.

Libina N, Berman JR, Kenyon S (2003). Tissue specific activities of *C. Elegans* Daf-16 in the regulation of lifespan. Cell., 115: 489-502.

Livesey G, Elia M (1995). Short –chain fatty acids as energy source in the colon: metabolism and clinical implications. In: Physiological and clinical aspects of short-chain fatty acids. Cummings JH, Rombeau JL, Sakata T, (eds.) Cambridge: Cambridge University Press, 1995: 432-481.

Lin K, Hsin H, Libina N, Kenyon S (2001). Regulation of the *Caenorhabditis Elegans* longevity protein Daf-16 by insulin/IGF-1 and germline signaling. Nat. Genet., 28: 139-145.

Lin S, Ford E, Haigis M, Liiszt G, Guarante L (2004). Calorie Restriction extends yeast lifespan by lowering the level of NADH. Gene. Dev., 18: 12-16.

Liu T, Liu PY, Marshall GM (2009). The critical role of the clas III histone deacetylase SIRT1 in cancer**.** Cancer Res., 69(5): 1702-1705.

Long A, Martin PS (1974). Death of American Ground Sloths. Science. 186(4164): 638-640.

Moorthy SN, Mathew G (1998). Cassava fermentation and associated changes in physiochemical and functional properties. Crit. Rev. Food Sci. Nutr., 38(2): 73-121.

Morcos M, Du X, Pfisterer F, Hutter H, Sayed AR, Thornalley P, Amen N, Baynes J, Thorpe S, Kukudov G, Schlotterer A, Bozorgmehr F, El Baki RA, Moehrlen F, Stern D, Ibrahim Y, Oikonomou D, Hamann A, Becker C, Zeier M, Schwenger V, Rabbani N, Fleming T, Zeier M,

Murphy CT, Lee S, Kenyon C (2007). Tissue entrainment by feedback regulation of insulin gene expression in the endoderm of *Caenorhabditis Elegans*. PNAS. 104(48): 19046-19050.

Nassar NA (1978). Conservation of the genetic resources of cassava (*Manihot esculenta*) determination of wild species localities with emphasis on probable origin. Econ. Bot., 32: 311-320.

Olsen KM (2004). SNP's SSR's and inferences on cassava's origin. Plant. Mol. Biol., 56: 517-526.

Olsen KM, Schall BA (1999). Evidence on the origin of Cassava: Phylogeography of *Manihot esculenta.* PNAS., 96: 5586-5591.

Olsen KM, Schall BA (2001). Microsatellite variation in cassava (*Manihot Esculenta*, Euphorbiaceae) and its wild relatives: further evidence for a southern origin of domestication. Am. J. Bot., 88(1): 131-142.

Parashar V, Rogina B (2009). dSir2 mediates the increased spontaneous physical activity in flies on calorie restriction. Aging. 1(6): 529-541.

Pedersen SB, Ølholm J, Paulsen SK, Bennetzen MF, Richelsen B (2008). Low Sirt1 expression, which is upregulated by fasting, in human adipose tissue from obese women. Int. J. Obes. (Lond.). 32(8): 1250-1255.

Pruitt K, Zinn RL, Ohm JE, McGarvey KM, Kang SL, Watkins DN, Herman JG, Baylin SB (2006). Inhibition of SIRT1 Reactivates Silenced Cancer Genes without Loss of Promoter DNA Hypermethylation. PLoS Genet., 2(3): e40.

Pujos F (2008). Paleogeographic distribution and anatomical adaptations in Peruvian Megatherium ground sloths (xenarthra: Megatheriodea). In Vizciano SF, Loughry WJ (eds.): The Biology of Xenarthra. Gainesville: University Press of Florida, pp. 56-63.

Pujos F, Salas R (2004). A systematic reassessment and paleographic review of fossil Xenarthra from Peru. Bulletin de l'Institut français d'études andines. Lima, 33(2): 331-377.

Roa AC, Chavarriaga-Aguirre P, Duque MC, Maya MM, Bonierbale MW, Inglesias C, Tohme J (2000). Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship^1. Am. J. Bot., 87(11): 1647-165.

Rogers DJ, Appan SG (1973). Manihot and Manihotoides (Euphorbiaceae). A computer assisted study. Hafner Press, New York

Rossetti DF, Toledo PM, Moraes-Santos HM, Araujo Santos AE (2004). Reconstructing habitats in Central Amazonia using megafauna, sedimentology, radiocarbon, and isotope analyses. Quaternary Res., 61: 289-300

Roy CC, Kien CL, Bouthillier L, Levy E (2006). Short-chain fatty acids: ready for prime time? Nutr. Clin. Pract., 21(4): 351-366.

Salvador LD, Suganuma T, Kitahara K, Fukushige Y, Tanoue H (2002). Degradation of cell wall materials from sweetpotato, cassava, and potato by a bacterial protopectinase and terminal sugar analysis of the resulting solubilized products. J. Biosci. Bioeng., 93(1): 64-72.

Sakakibara I, Fujino T, Ishii M, Tanaka T, Shimosawa T, Miura S, Zhang W, Tokutake Y, Yamamoto J, Awano M, Iwasaki S, Motoike T, Okamura M, Inagaki T, Kita K, Ezaki O, Naito M, Kuwaki T, Chohnan S, Yamamoto TT, Hammer RE, Kodama T, Yanagisawa M, Sakai J (2009). Fasting-induced hypothermia and reduced energy production in mice lacking acetyl-CoA synthetase 2. Cell Metab., 9(2): 191-202.

Schall BA, Olsen KM, Carvalho LJBC (2006). Evolution, domestication, and agrobiodiversity in the tropical crop Cassava. In: Motley T, Zerega N, and Cross H, (eds.): Darwin's Harvest, Columbia University Press, pp. 269-284.

Schlotterer A, Kukudov G, Bozorgmehr F, Hutter H, Du X, Oikonomou D, Ibrahim Y, Pfisterer F, Rabbani N, Thornalley P, Sayed AR, Fleming T, Humpert M, Schwenger V, Zeier M, Hamann A, Stern D, Brownlee M, Bierhaus A, Nawroth P, Morcos M (2009). C. Elegans as model for the study of high glucose-mediated lifespan reduction. Diabetes. 58: 2450-2456.

Scher MB, Vaquero A, Reinberg D (2007). SirT3 is a nuclear NAD+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. Gene. Dev., 21(8): 920-928.

Schwer B, Bunkenborg J, Verdin RO, Andersen JS, Verdin E (2006). Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. PNAS., 103(27): 10224-10229.

Shimazu T, Hirschey MD, Huang JY, Ho LT, Verdin E (2010). Acetate metabolism and aging: An emerging connection. Mech. Ageing Dev., 131(7-8): 511-516.

Shogren-Knaak M, Ishii H, Sun JM, Pazin MJ, Davie JR, Peterson CL (2006). Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science, 311(5762): 844-847.

Singer S, Souza K, Thilly WG (1995). Pyruvate utilization, phosphocholine and adenosine triphosphate (ATP) are markers of human breast tumor progression: a 31P- and 13C-nuclear magnetic resonance (NMR) spectroscopy study. Cancer Res., 55(22): 5140-5145.

Skutches CL, Holroyde CP, Myers RN, Paul P, Reichard GA (1979). Plasma Acetate turnover and oxidation. J. Clin. Invest., 64: 708-713.

Stark AH, Madar Z (1993). *In vitro* production of short-chain fatty acids by bacterial fermentation of dietary fiber compared with effects of those fibers on hepatic sterol synthesis in rats. J. Nutr., 123(12): 2166-2173.

Steadman DW, Martin PS, MacPhee RDE, Jull AJT, McDonald HG, Woods CA, Iturralde-Vinent M, Hodgins GWL (2005). Asynchronous extinctions of late Quaternary sloths on continents and islands. PNAS., 102: 11763-11768.

Tessari P, Nissen SL, Miles JM, Haymond MW (1986). Inverse relationship of leucine flux and oxidation to free fatty acid availability *In vivo*. J. Clin. Invest., 77(2): 575-581.

Tewe OO (2004). Cassava for livestock feed in sub-Saharan Africa in The Global Cassava Development Strategy, Food and Agriculture Organization of the United Nations, http://www.fao.org/docrep/007/j1255e/j1255e00.htm

Thangaraju M, Gopal E, Martin PM, Ananth S, Smith SB, Prasad PD, Sterneck E, Ganapathy V (2006). SLC5A8 triggers tumor cell apoptosis through pyruvate-dependent inhibition of histone deacetylases. Cancer Res., 66(24): 11560-11564.

Thangaraju M, Carswell KN, Prasad PD, Ganapathy V (2009). Colon cancer cells maintain low pyruvate to avoid cell death caused by inhibition of HDAC1/HDAC3. Biochem. J., 417: 379-389.

Tito G, De Iuliis G (2003). Morphological aspects and paleobiology of the Manus in the Giant Ground Sloth *Eremotherium* Spillman 1948 (Mammalia, Xenarthra, Megatheriidae). Senckenbergiana biologica, 83: 79-94.

Vizciano SF, Bargo SM, Farina FA (2008). Form function and paleobiology in Xenarthrans. In Vizciano SF, Loughry WJ (eds.): The Biology of Xenarthra. Gainesville: University Press of Florida, pp. 86-99.

Weber J, Kayser A, Rinas U (2005). Metabolic flux analysis of *Escherichia coli* in glucose-limited continuous culture. II. Dynamic response to famine and feast, activation of the methylglyoxal pathway and oscillatory behavior. Microbiology, 151: 707-716.

Weed J L, Lane M A, Roth G S, Speer D L, Ingram D K (1997). Activity Measures in Rhesus Monkeys on Long-Term Calorie Restriction. Physiol. Behav., 62: 97-103.

Yeoh HH, Chew MY (1976). Protein content and Amino acid content of cassava leaf. Phytochem., 15: 1597-1599.

Yeoh HH (1996). Protein contents, amino acid compositions and nitrogen-to-protein conversion factors for cassava roots. J. Sci. Food Agric., 70: 51-54.